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Award Number: DAMD17-02-1-0561

TITLE: Endothelial Cell-Targeted Adenoviral Vector for
Suppressing Breast Tumors

PRINCIPAL INVESTIGATOR: Shuang Huang, Ph.D.

CONTRACTING ORGANIZATION: The Scripps Research Institute
La Jolla, California 92037

REPORT DATE: April 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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20030904 083

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 074-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE April 2003	3. REPORT TYPE AND DATES COVERED Annual (1 Apr 02 - 31 Mar 03)		
4. TITLE AND SUBTITLE Endothelial Cell-Targeted Adenoviral Vector for Suppressing Breast Tumors		5. FUNDING NUMBERS DAMD17-02-1-0561		
6. AUTHOR(S): Shuang Huang, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Scripps Research Institute La Jolla, California 92037 E-Mail: shuang@scripps.edu		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSORING / MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. Abstract (Maximum 200 Words) <i>(abstract should contain no proprietary or confidential information)</i> Angiogenesis is essential for the growth and metastasis of solid tumors including breast cancer. In vitro and in vivo experimental models clearly demonstrate that suppressing angiogenesis leads to tumor suppression. The overall goal of this proposal is to develop an adenovirus-based gene therapy approach for suppressing angiogenesis. In the first year of the funding period, we focused our effort on developing the endothelial cell-targeted adenovirus vector. We incorporated five previously published endothelial cell-specific peptide sequences into adenovirus capsid fiber sequence and the modified fibers were added to β -galactosidase-containing adenovirus with a helper-cell strategy. We examined the infectivity and specificity on human microvascular endothelial cells and several other cell lines and found that two of the published sequences (NGR and SPARC) were able to facilitate adenoviral vectors specifically and efficiently transducing human endothelial cells. These studies clearly demonstrate that endothelial cell-targeted adenoviral vector can be generated rather simply and easily. In our future studies, we will use our developed vector to deliver anti-angiogenesis genes to tumor vascular structure and to evaluate the efficacy of these vectors to suppress breast tumor development in both in vitro and in vivo models.				
14. SUBJECT TERMS: angiogenesis, gene therapy, adenoviral vector, endothelial cells			15. NUMBER OF PAGES 7	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

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Introduction

The survival, growth and metastasis of solid tumors including breast cancer depends on the formation of new blood vessels to provide tumors with nutrients and oxygen, a process called angiogenesis. *In vitro* and *in vivo* experimental models indicate that suppressing angiogenesis can also suppress solid tumors. However, the success of this approach largely depends on whether sufficient amounts of therapeutic agents can be delivered to tumor-associated endothelial cells without causing toxic effect to other tissues/cells. Our proposal is designed to develop an endothelial cell-targeted adenoviral vector and to use the targeted vector to express high levels of anticancer therapeutic genes in the sites of angiogenic tumors specifically and efficiently.

Body

After I submitted this grant proposal, several more peptide sequences have been reported being tumor-associated endothelial cell-specific. In the first year of the funding period, we thus also tested the specificities of these peptides for endothelial cell targeting in addition to our previously proposed NGR and GSL peptides. We first synthesized oligonucleotides encoding for CGSLVRC (GSL in short) (1), CNGRCSVSGCAGRC (NGR in short) (2), TCDLDNDKYIALEEWAGCFG (SPARC in short) (3), EQRLGNQWARGHLM (GRP in short) (4), and RGD peptides (2). These oligonucleotides were cloned into pDV137 plasmid. The pDV136 plasmid has two major features. 1) This plasmid contains a cloning site (Bsp E1) in HI loop of fiber gene to allow insertion; and 2) Two point mutations was introduced in the fiber gene so that the expressed is no longer able to interact with cells through the cell surface adenovirus receptor (CAR) (5). The resulted plasmids were individually transfected into 293 cells and the stable transfectants were collected. We analyzed the expression of the modified fiber and the trimerization of fiber (Fig.1) and found that the insertion of peptide sequence in the HI loop of the fiber gene did not affect fiber expression and trimerization in 293 cells. These results suggest that it is possible to use a helper-cell system to trans-supply fiber to adenovirus constructs.

In the next experiment, we constructed a fiber-deleted, β -galactosidase gene containing adenoviral vector. This was done first by deleting the region encoding fiber gene from pAd.Easy-1 (Q-Biogene), and then by cloning β -galactosidase gene into pShuttle/CMV (Q-Biogene). The generated plasmids were electroporated in recombination-competent BJ5183 E.coli strain and the resulted colonies were analyzed for correct adenovirus recombination. The plasmid with correct recombination was transfected into 203 cells with various fiber constructs. The resulted adenoviral constructs are 1) fiberless Ad, 2) Ad with wild-type fiber (Ad-wt), 3) Ad with fiber containing GSL

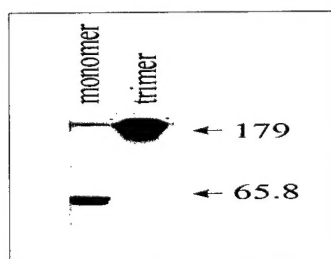


Fig.1. The insertion of peptide sequence in the HI Loop of Ad fiber does not affect fiber trimerization. NGR-inserted fiber expression vector was transfected into 293 cells. The transfected cells were lysed and the cell lysates were either boiled or not boiled prior to electrophoresis. The fiber expression was detected with anti-fiber polyclonal antibody. The trimerized fiber can be clearly observed in non-boiled sample.

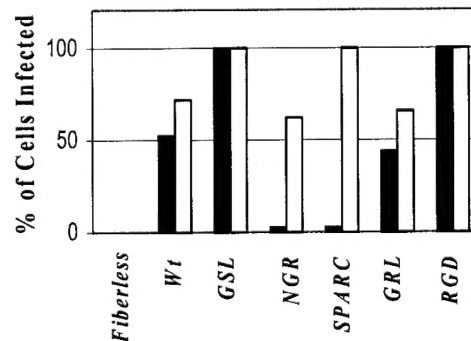


Fig.2. NGR- and SPARC-Ad selectively deliver gene in human endothelial cells. HeLa (closed bars) and HMVCAD cells (open bars) were infected with various β -gal containing Ad vector (10pfu/cell, 100viral particle/cell for Fiberless Ad) for 24 hrs. The infectivity of these viruses was determined by detecting β -galactosidase activity.

peptide (GSL-Ad), 4) Ad with fiber containing NGR peptide (NGR-Ad), 5) Ad with fiber containing SPARC peptide (SPARC-Ad), 6) Ad with fiber containing RGD (RGD-Ad),

and 7) Ad with fiber containing GRL peptides (GRL-Ad). To determine the infectivity and specificity of these viruses, HeLa and HMVCAD were infected with these viruses at 10pfu/cell (fiberless Ad was used at 100 viral particles/cell) for 48 hrs and the infection was determined by detecting counting cells with β -galactosidase activity. As shown in Fig.2, fiberless Ad showed no infectivity to both lines suggesting fiber is required for virus infection. RGD-Ad and GRL-Ad displayed similar infectivity to Ad-wt in both lines. GSL-Ad showed better infectivity than Ad-wt in both lines. Interestingly, NRG-Ad and SPARC-Ad exhibited great infectivity to HMVCAD but showed little infectivity to HeLa cells. To further confirm the specificity of NRG-Ad and SPARC-Ad to endothelial cells, we also infected JURKAT T cells, THP1 monocytic cells, A549 lung carcinoma cells, PC3 prostate cancer cells and human foreskin fibroblast cells with these two viruses and found that all these lines were either not or only slightly infectable by NRG-Ad or SPARC-Ad (data not shown). These results suggest that we have obtained endothelial cell-specific Ad delivery vector.

Key Research Accomplishment:

- We have successfully developed a truly endothelial cell-targeted adenoviral vector.

Reportable Outcomes

The preliminary data generated from the last funding year have been used in a newly submitted grant entitled "Combating radiation resistance in prostate cancer" to the Department of Army Prostate Cancer Research Program.

Conclusions

The studies performed in the 1st year funding period demonstrate that the endothelial cell-targeted adenoviral vectors can be generated rather simply and fast. This finding is important since the major obstacle for adenovirus-based gene therapy is lack of specificity and transduction to the targeted tissues/cells.

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Appendices

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